

FIG. 1

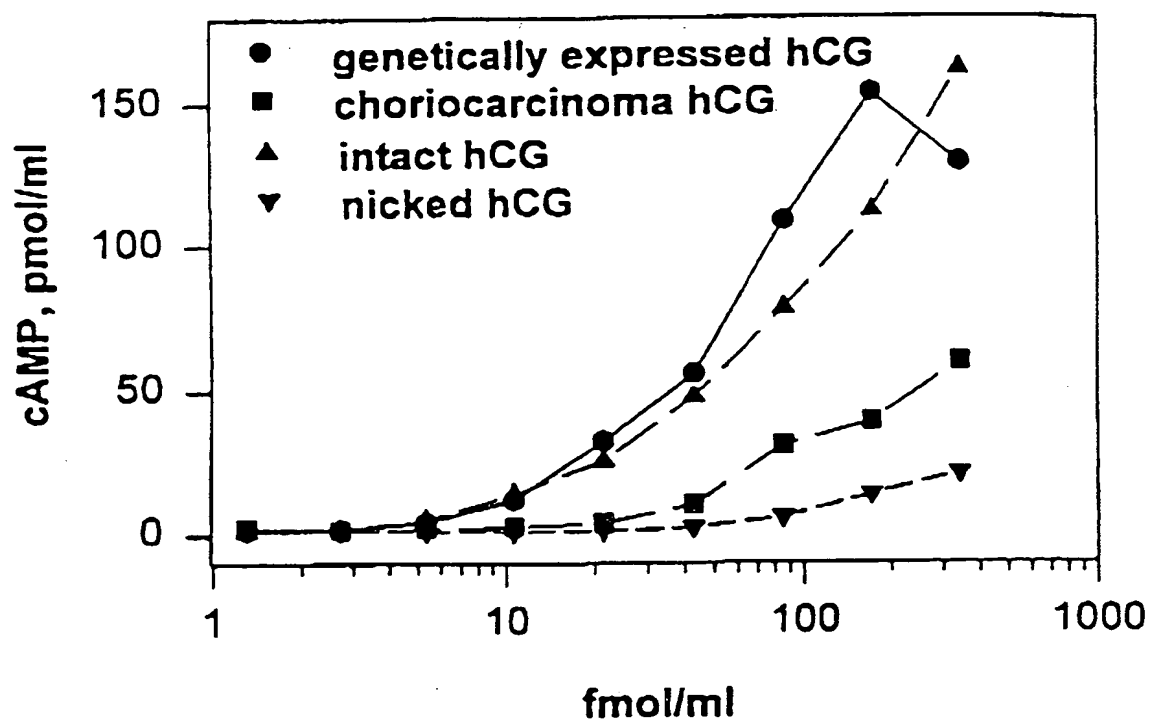


FIG. 2A

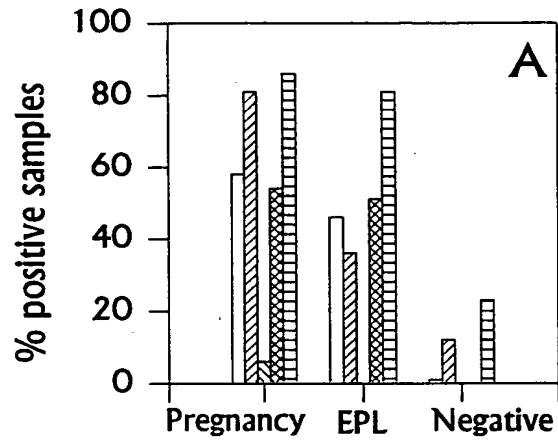
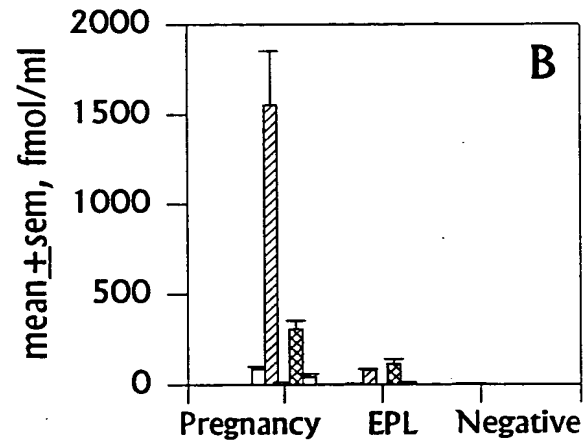
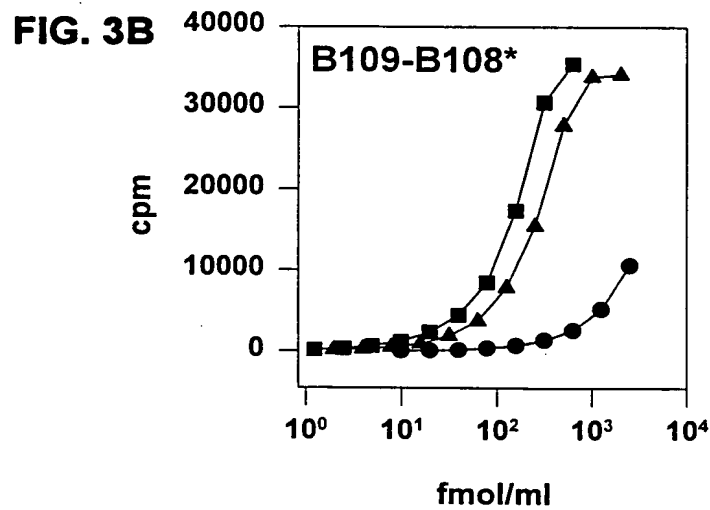
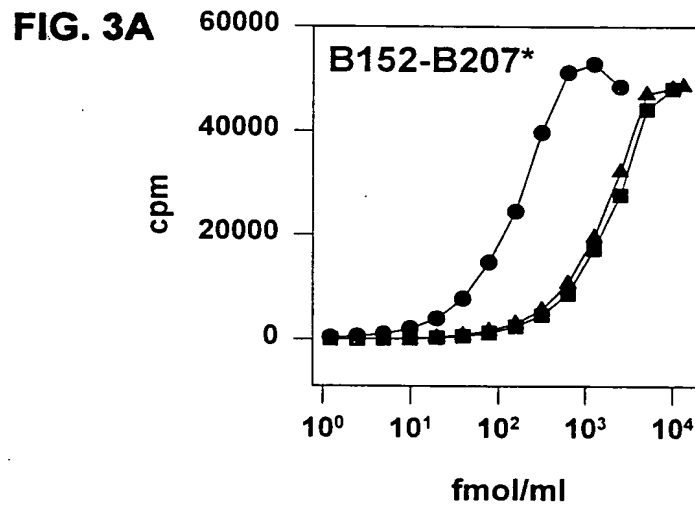


FIG. 2B

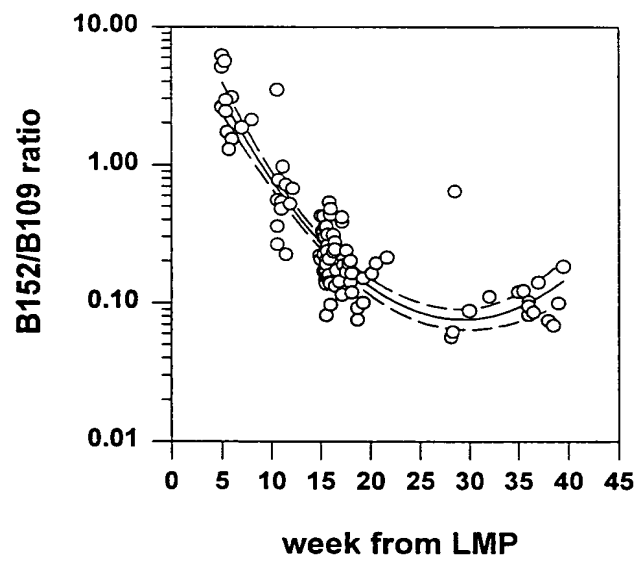


hCG (B109-B108*)
hCG (B152-B207*)
nhCG (B151-B207*)
hCG β (B201-C104*)
hCG β cf (B210-B108*)



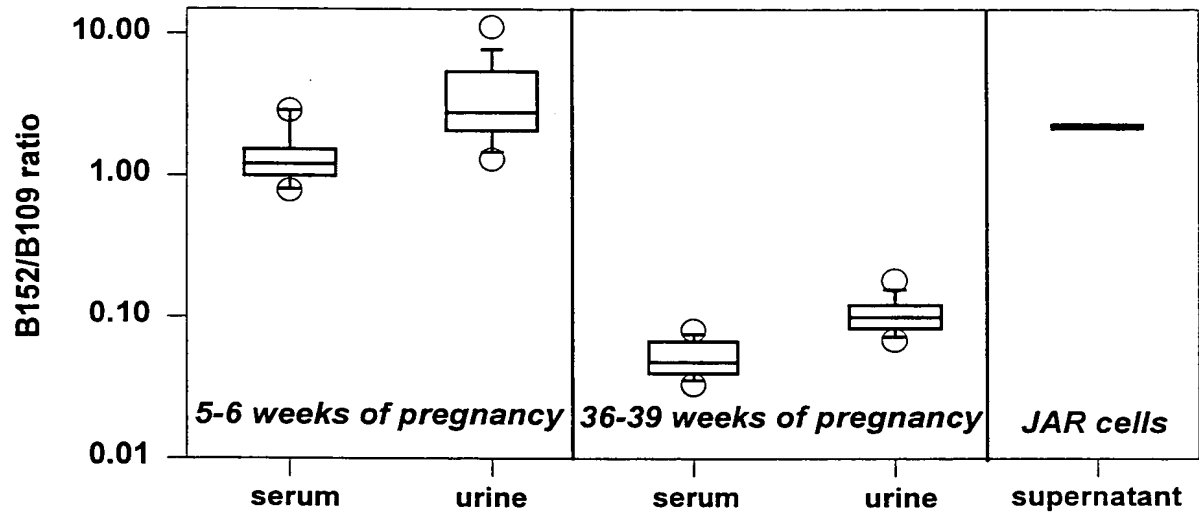
- choriocarcinoma hCG (C5)
- pregnancy hCG
- ▲ pituitary hCG

FIG. 4



1992-1993-1994-1995

FIG. 5



6/15

FIG. 6

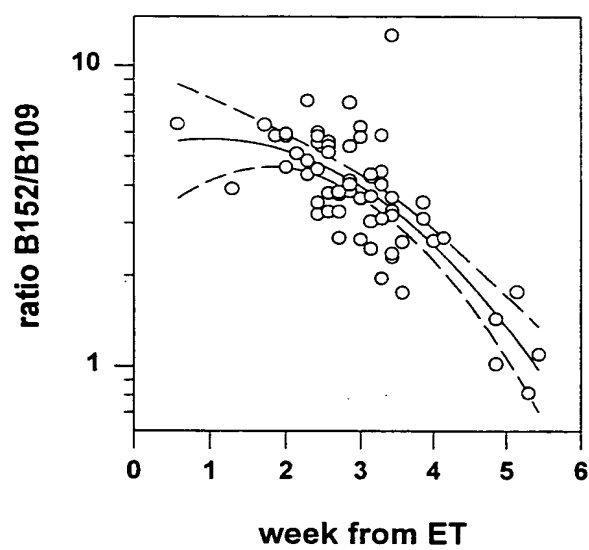


FIG. 7A

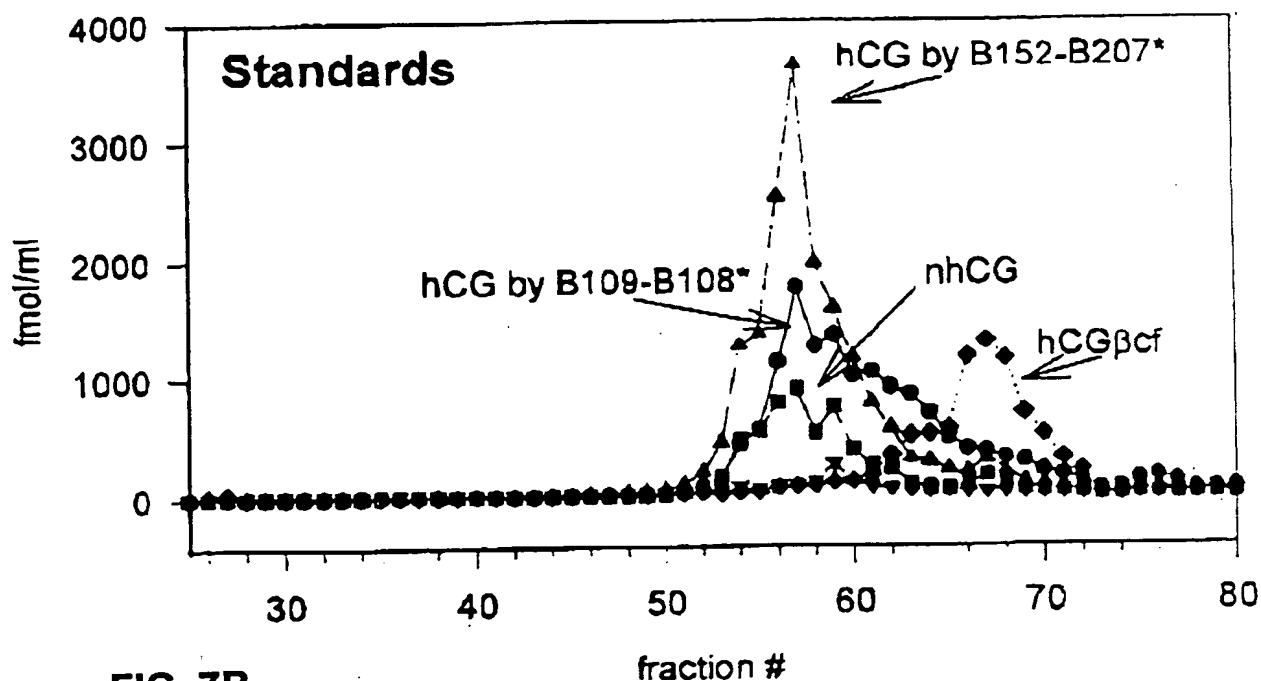


FIG. 7B

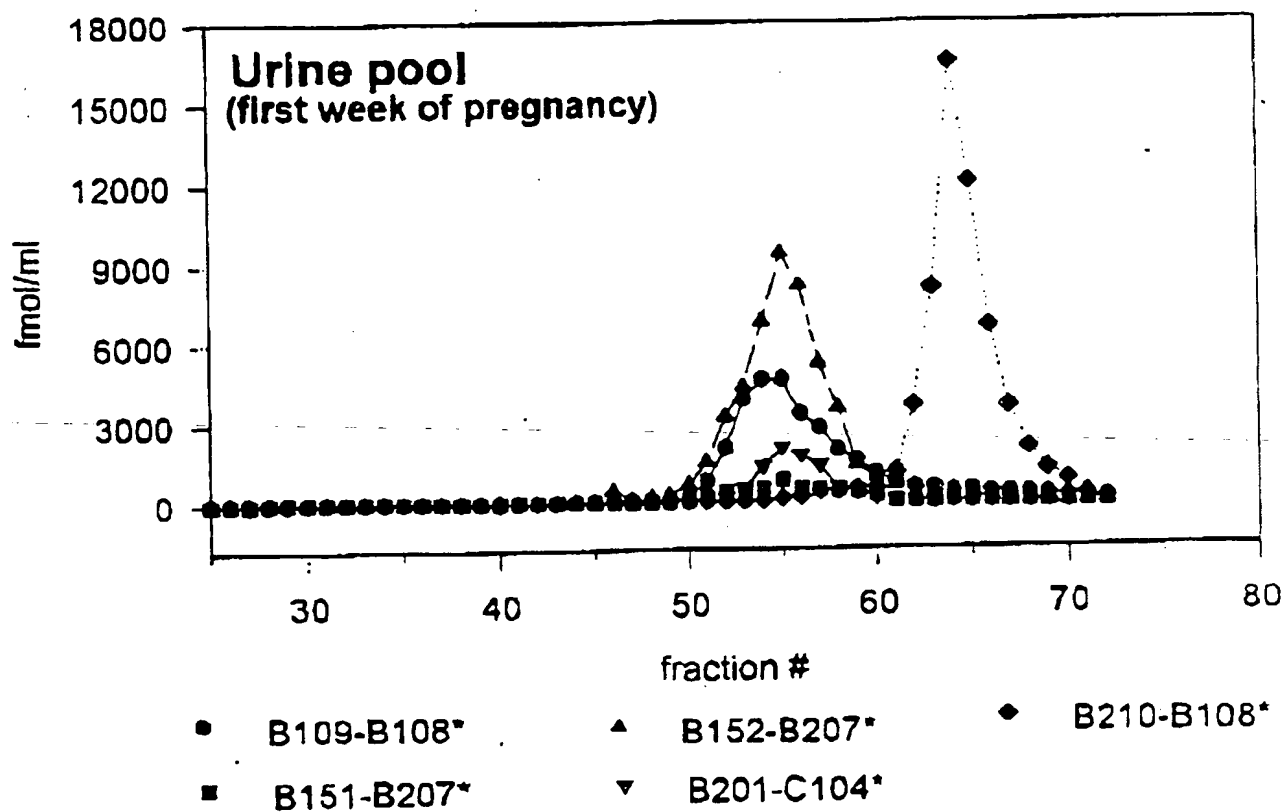


FIG. 8A

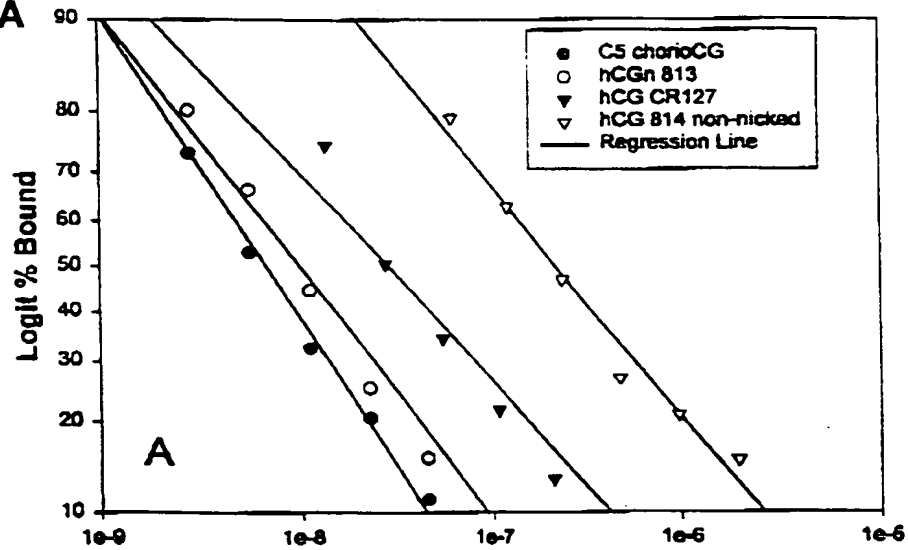


FIG. 8B

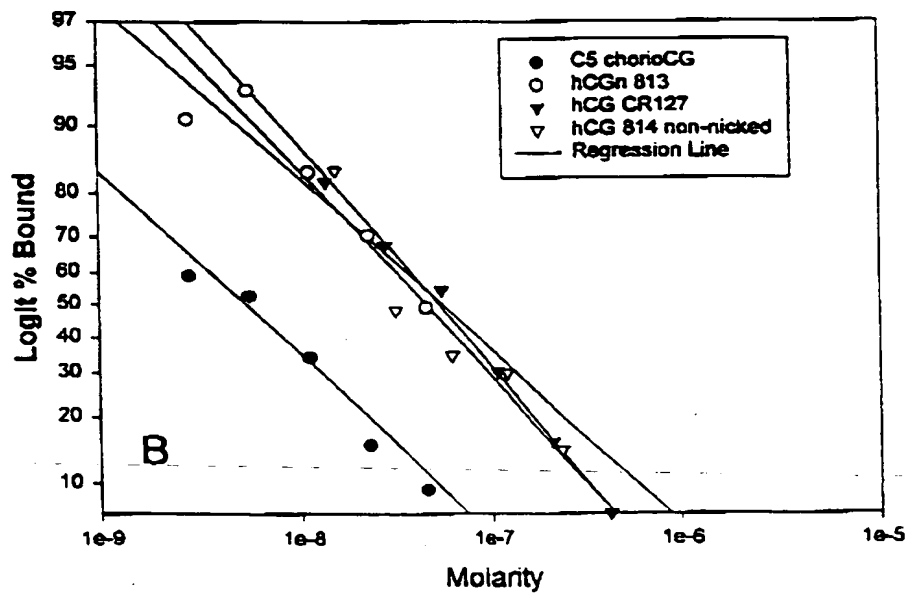


FIG. 9A

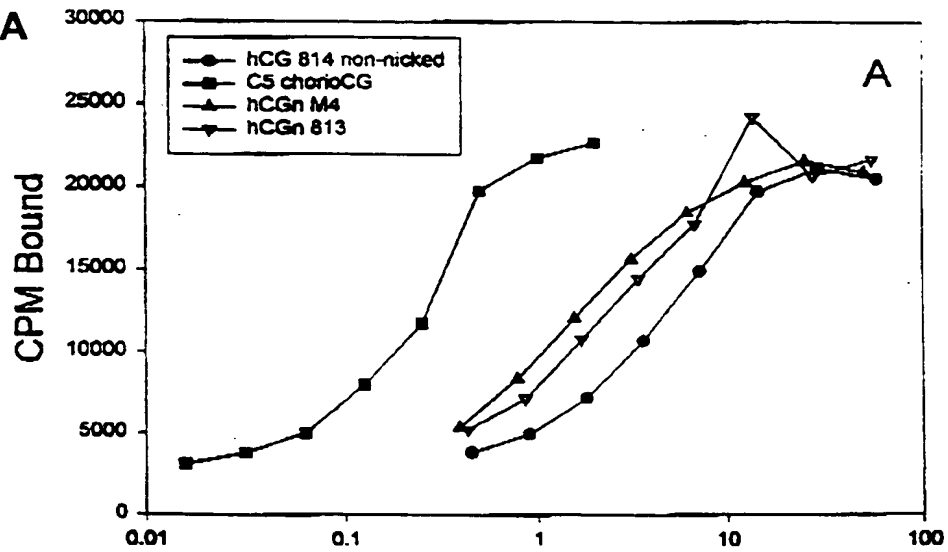


FIG. 9B

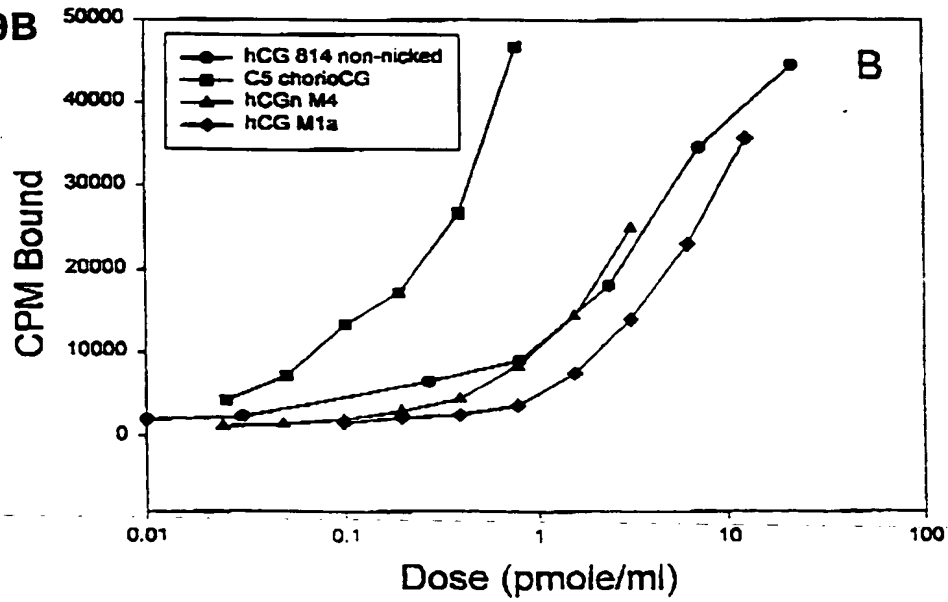


FIG. 10

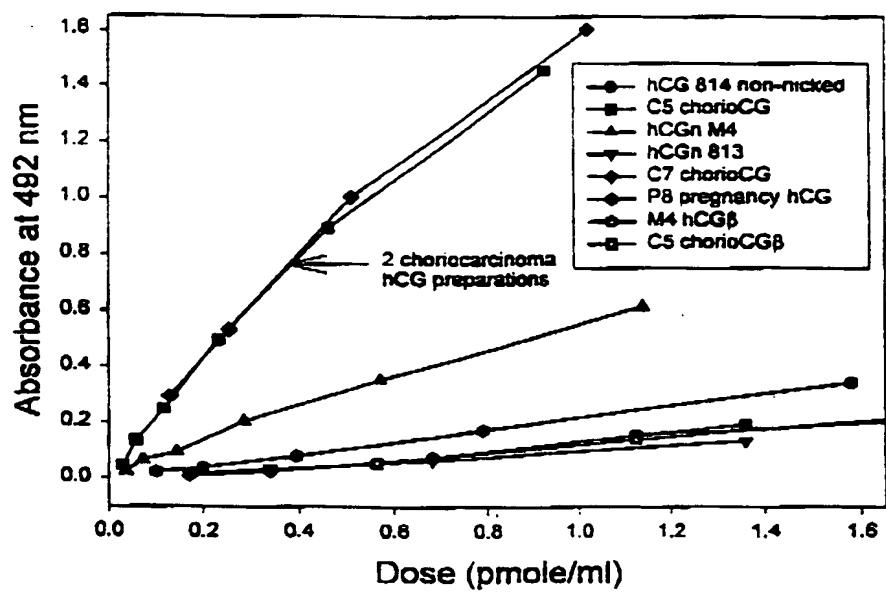


Table I. Characteristics of the Reagents Used to Define Antibody Specificity. The peptide and carbohydrate structures of the reagents used were determined earlier (26). The % nicked β -subunit refers to the proportion of molecules with cleavages (missing peptide bonds) in the region β 43 to β 48. The % tetrasaccharide core is the proportion of O-linked oligosaccharides with tetrasaccharide (vs. disaccharide) core structure, and the % sialic acid, is the proportion of O-linked structures with antennae terminated by sialic acid residues. The proportion of triantennary N-linked oligosaccharides on β -subunit is given, as is the corresponding % sialic acid.

^a % sialic acid residues per sugar chain, N-linked on β .
^b % sialic acid residues per sugar chain, O-linked on β .
^c The "CR" series of hCG reference preparations were made at Columbia University and were distributed internationally as reference materials for purified hCG. CR 119 is also known as the 3rd international immunoassay reference preparation for hCG.
^d ND is not done; NA is not applicable to that reagent.
^e Less than 15% of the beta COOH-terminal region is present on this preparation.

FIG. 12

Table II: Affinity Constants^a Determined by Liquid Phase Competition Assays Using C5 as Tracer Ligand

Antibody	Competitors			
	C5 chorioCG	Nicked hCG CR 127 (813)	Parent CR 127 hCG ^b	Nick-free hCG CR127 (814)
B151	4.4×10^8	3.8×10^8	4.2×10^7	1.3×10^7
B152	3.5×10^8	5.4×10^7	4.7×10^7	5×10^7

^aK_a as LM

^b hCG CR 127 is an NIH-distributed hCG reference preparation produced at Columbia University.

FIG. 13A

Table III: Matrices of data for binding characteristics of different pairs of detection antibodies using B151 or B152 as capture antibody.

A. Relative Cross-Reactivities of Two Site Assay Using B151 as Capture Antibody

Ligand	B207 ^{a,c}	B204 ^a	B201 ^a	B108 ^a	B109 ^a	A109 ^a	CTP104
C5	100%	< ^b	<	100%	<	<	100
813 CR 127 hCGn	100%	<	<	100%	<	<	47%
814 CR127 hCG	12%	<	<	37%	<	<	14%
HCG β	2%	<	<	2%	<	<	<
C5 β	5%	<	<		<	<	<
HCG β core	<	<	<		<	<	<
HLH	2%	<	<	3%	<	<	<
HLH β	5%	<	<		<	<	<
HCG α	<	<	<	3%	<	<	<
Maximum binding ^b	50%	0%	0%	13%	0%	0%	83%

^a labeled detection antibodies

^b cut of low range detection

^c this particular assay format was applied in O'Connor et al (25).

FIG. 13B

B. Relative Cross-Reactivities of Two Site Assay Using B152 as Capture Antibody

Ligand	B207 ^{a,d}	B204 ^a	B201 ^a	B108 ^a	B109 ^a	A109 ^a	CTP104 ^a
C5	100%	100%	94%	42%	53%	100%	<
813 CR 127 hCGn	10%	< ^b	<	15%	32%	64%	<
814 CR127 hCG	7%	<	<	30%	100%	26%	<
HCG β	6%	20%	19%	11%	<	<	<
C5 β	190%	100%	100%	100%	<	<	<
HCG β core	<1%	<	<	<	<	<	<
HLH	<1%	<	<	<	<	<	<
hLH β	<1%	<	<	<	<	<	<
HCG α	<1%	<	<	<	<	<	<
Maximum binding ^b	64%	2%	44%	80%	2%	14%	25%

The molar quantity of ligand required to produce binding equal to 50% of the maximum binding achieved by C5 was determined. Cross-reactivity shown in this table as a percentage is calculated by dividing the molar quantity of the standard by the molar quantity of the other ligand at 50% maximum binding dose.

^a labeled detection antibodies

^b maximum binding represents the total quantity of radiolabeled detection antibody which can bind to the plate in the system described.

^c < out of low range detection

^d this particular assay format was applied in O'Connor et al (25).

FIG. 14

Table IV. Immunoreactivity of antigens in the B152 immunoradiometric assay. The dose-response curves used to provide data for this table are shown in Figure 3. Each curve was fitted with 4-5 points. Slope and coefficient of determination (R^2) were determined using a non-linear regression algorithm. Slopes were used as an indicator of antigen potency. Relative potency was estimated as the slope of antigens relative to the slope of C5 Choriocarcinoma hCG (the immunogen).

Reagent	Slope *	S.E.	R^2	Relative Potency
814 hCG	0.1588	0.0098	0.992	10.2%
C5 chorioCG	1.5603	0.1015	0.983	100%
M4 mole hCGn	0.5317	0.0240	0.992	34%
813 hCGn	0.0986	0.0021	0.999	6.3%
C7 chorioCG	1.4515	0.1246	0.985	93.0%
P8 hCG	0.2192	0.0031	0.999	14.0%
M4 mole hCG β	0.1038	0.0069	0.991	6.67%
C5 chorioCG β	0.1286	0.0042	0.998	8.24%
CR 129 hCG β	Only 2 points			
HLH batch I-1	No response			

* Slope are from figure 3 as calculated in Sigmaplot 4.01 by linear regression analysis. Units of slope are pmole/ml absorbance at 492nm.